

Applicants: Robert J. Winchester, et al.
Serial No.: 09/500,746
Filed : February 9, 2000
Page 2

In the Specification:

Please amend the paragraphs of the specification identified below with the amended versions thereof as follows:

The paragraph on page 15, line 25 through page 16, line 35:

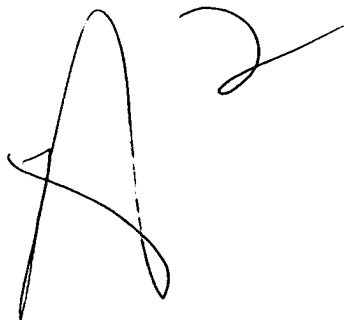
(Amended) **Construction of the subtraction library and preliminary sequencing.** PolyA⁺ RNA was isolated from the fifth passage synoviocytes using a mRNA Isolation Kit (Stratagene). 2ug of twice purified polyA⁺ RNA was used as a template for cDNA synthesis in the RiboClone cDNA Synthesis System (Promega). The synthesized cDNA was ligated with the oligonucleotides GATCCGCGGCCGC (SEQ ID NO:1) and GCGGCCGCGT (SEQ ID NO:2) as described (Hubank 1994). After selection of fragments larger than 250 nucleotides by fractionation through a Sephacryl S-400 column (Pharmacia) and phosphorylation with T4 polynucleotide kinase, the cDNA was digested with the restriction enzyme MboI. The fragments were then ligated with oligonucleotides J-Bam-24 ACCGACGTCGACTATCCATGAACG (SEQ ID NO:3) and J-Bam-12 GATCCGTTCATG (SEQ ID NO:4), and amplified as described (Hubank 1994). The PCR products, after fractionation through Sephacryl S-400 column, were digested with MboI and they comprised the primary amplicon. DNA from rheumatoid arthritis synoviocytes was further ligated with oligonucleotides N-Bam-24 AGGCAACTGTGCTATCCGAGGGAG (SEQ ID NO:5) and N-Bam-12 GATCCTCCCTCG (SEQ ID NO:6). The hybridization was performed as described (26) except that the ratio of tester and driver was kept 1:100 throughout. 10ug of the osteoarthritis primary amplicon were hybridized with 0.1ug of the rheumatoid arthritis primary amplicon in 5ul of 24mM EPPS, pH8.0, 1mM EDTA, 1M NaCl for 20hr at 67C. The hybridized DNA was subjected to 10 cycles of PCR with N-Bam-24 as a primer, followed by digestion with mung bean nuclease. One four hundredth of the digests was further

Applicants : Robert J. Winchester, et al.
U.S. Serial No.: 09/500,746
Filed : February 9, 2000
Page 3

amplified for 20 cycles. After digestion with MboI, the DNA product was ligated with oligonucleotides R-Bam-24 AGCACTCTCCAGCCTCTCACCGAG (SEQ ID NO:7) and R-Bam-12 GATCCTCGGTGA (SEQ ID NO:8). Hybridization and amplification steps were repeated. After redigestion with MboI, the resulting differentially subtracted cDNA fragments were cloned into a BamHI site of the plasmid pUC18. The recombinants were inoculated in an ordered grid pattern on nitrocellulose filters, which were then probed with the osteoarthritis cDNA amplicon ³²P-labeled with the Megaprime DNA labeling System (Amersham). The DNA sequence of the non-hybridized recombinants was determined in an Applied Biosystems DNA Sequencer Model 373A or 377 using standard dye terminator chemistry. The seqman module of the Lasergene program (DNASTar) was used for identification of homologous recombinants and grouping them into groups. The Genman module of the Lasergene program was used to search the GenBank databases including the expressed sequence tag database on CDROM. BLAST was used to verify the identification of sequences that showed no homology with entries in the CDROM database.

The paragraph on page 6, line 7 through line 18:

(Amended) Figure 2 Comparison of the amino acid sequence of human semaphorin III (SEQ ID NO:13), IV (SEQ ID NO:11), V (SEQ ID NO:12), and mouse semaphorin E (SEQ ID NO:9), with the predicted sequence of human semaphorin VI (SEQ ID NO:10). Nucleotide sequence of the cDNA fragment of human semaphorin VI was translated into an amino acid sequence, and compared to that of the corresponding region of human semaphorin III, IV, V and mouse semaphorin E. Conserved amino acids are indicated with boxes. One amino acid gap



Applicants : Robert J. Winchester, et al
U.S. Serial No.: 09/500,746
Filed : February 9, 2000
Page 4

introduced in the human semaphorin III and V to obtain the best alignment was marked by X.

The paragraph on page 6, line 7 through line 18:

A3
(Amended) Figure 3 Comparison of amino acid sequence of the human N-acetylglucosamine-6-sulfatase (SEQ ID NO:16) and predicted amino acid sequence from the *C. elegans* cosmid K09C4 (SEQ ID NO:14) and the clone ts99 (SEQ ID NO:15). Nucleotide sequence of the cDNA fragment of the clone ts99 was translated to an amino acid sequence, and the corresponding region of the human N-acetylglucosamine-6-sulfatase and *C. elegans* cosmid K09C4 were compared. Conserved amino acids are marked with boxes.

The paragraph on page 8, line 17:

A4
(Amended) Figure 12 SDF-1 Sequence (SEQ ID NO:17), (SEQ ID NO:18), (SEQ ID NO:19), (SEQ ID NO:20), (SEQ ID NO:21), (SEQ ID NO:22) and (SEQ ID NO:23).

REMARKS

Pursuant to the requirements of 37 C.F.R. §1.121, applicant annexes hereto as Exhibit D those paragraphs amended herein marked up to show the changes made herein relative to the previous versions thereof.

In the June 25, 2001 Notice, the Examiner stated that the application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences